[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] XXXII. Analogs of Chlorambucil. II. Monofunctional Alkylating Agents Derived from 3-(*p*-Aminophenyl)propionic Acid

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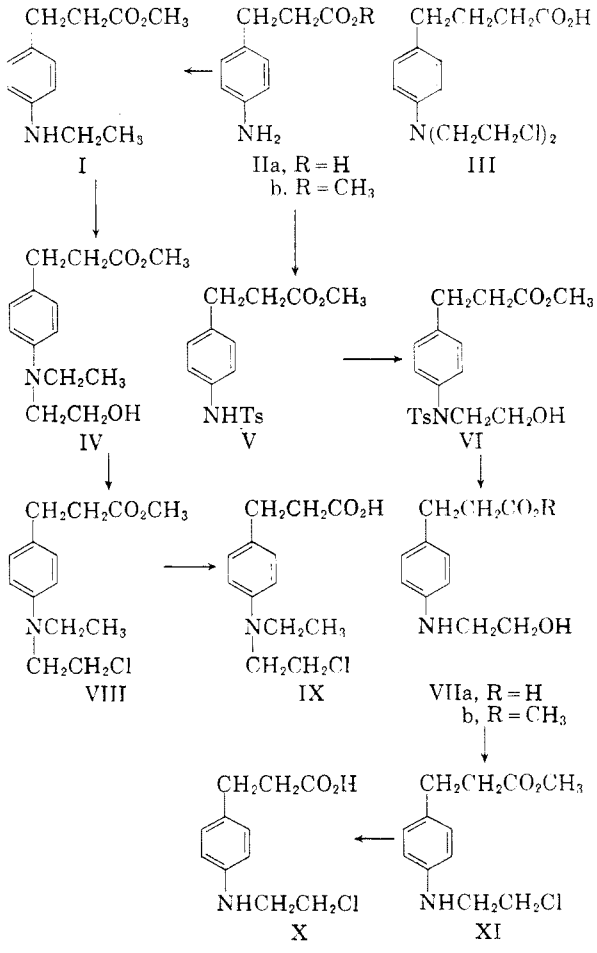
Four compounds containing monofunctional alkylating groups have been derived from 3-(*p*-aminophenyl)propionic acid, namely, 3-{*p*-[(2-chloroethyl)ethylamino]phenyl}propionic acid (IX), 3-[*p*-(2-chloroethylamino)phenyl]propionic acid (X), 3-(*p*-diazoniumphenyl)propionic acid bisulfate (XII), and methyl 3-[*p*-(*N*-nitrosoacetamido)phenyl]propionate (XIV)

It has been suggested[2] that anticancer agents consisting of an alkylating group on the proper carrier may function by selective irreversible inhibition of an enzyme system. If such a mechanism of irreversible inhibition is responsible for the activity of these agents, then one would suspect that a difunctional alkylating agent is not necessary for activity and that metabolites (carriers) containing a monofunctional alkylating group could also be effective.

Everett, *et al.*,[3] found that a series of *p*-[bis(2-chloroethyl)aminophenyl]carboxylic acids inhibited the growth of the transplanted Walker rat Sarcoma 256. The most active compound in the series was the butyric acid derivative, chlorambucil (III). The methyl and ethyl esters were also active. The activity of these compounds suggested testing the hypothesis of irreversible inhibition by the synthesis of chlorambucil analogs containing monofunctional alkylating groups. The choice of 3-phenylpropionic acid as a carrier moiety for an alkylating group in place of the somewhat more active 3-phenylbutyric acid carrier was based on the ready availability of the 3-phenylpropionic acid derivatives from the substituted cinnamic acids.

Recently,[4] a series of "one-armed" mustards derived from DL-phenylalanine were prepared and shown to be inactive against the Walker rat carcinoma 256. These results would indicate that phenylalanine mustards are not acting as selective irreversible inhibitors for Walker 256. This, however, does not rule out the possibility that the one-armed mustards derived from DL-phenylalanine might be more effective than the two-armed phenylalanine mustard on other tumors or that other one-armed mustards having a different carrier group could function as suggested.

3-{*p*-[(2-Chloroethyl)ethylamino]phenyl}propionic acid (IX) was synthesized in four steps from methyl 3-(*p*-aminophenyl)propionate (IIb). Reductive alkylation of IIb with acetaldehyde, hydrogen, and Raney nickel according to Emerson[5,6] yielded methyl 3-(*p*-ethylaminophenyl)pro-



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(2) H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3103 (1959).

(3) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).

(4) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 90 (1959).

(5) W. S. Emerson, in *Org. Reactions*, R. Adams, ed., John Wiley and Sons, Inc., New York, N. Y., 1948, Vol. IV, p. 174.

(6) W. S. Emerson and P. M. Walters, *J. Am. Chem. Soc.*, **60**, 2023 (1938).

pionate (I) in yields that varied from 46% to 61%. In order to obtain these higher yields, it was found necessary to keep the molar ratio of amine to acetaldehyde close to 1:1. Increasing the ratio of aldehyde to amine led to increased amounts of the diethylamino derivative being formed. Compound I was obtained as a solid, m.p. 35–36°, which traveled as a single spot (R_f 0.72) on acetylated paper[7] when detected by ultraviolet light or *t*-butyl hypochlorite spray[8] (violet color) for NH. The 3-[*p*(diethylamino)phenyl]propionate had an R_f of 0.58 in the same system and gave a negative *t*-butyl hypochlorite color test.

The preparation of methyl 3{-*p*-[ethyl(2-hydroxyethyl)amino]phenyl}propionate (IV) from I and ethylene oxide in acetic acid solution proceeded smoothly in 90% yield. The product was homogeneous on paper[7] (R_f 0.83).

The synthesis of methyl 3-{*p*-[(2-chloroethyl)-ethylamino]phenyl}propionate (VIII) from IV was accomplished in 61% yield, chlorination being effected by the use of thionyl chloride in chloroform. The product was purified by chromatography on a column of acid-washed alumina by elution with chloroform to yield the free base (VIII). This treatment removed colored by-products and allowed VIII to be obtained as an analytically pure, light yellow oil.

In larger-scale preparations of VIII, the reaction product could be purified more conveniently by stirring a chloroform solution of it with alumina to remove the pigmented by-products.

The desired final product, 3-{*p*-[(2-chloroethyl)-ethylamino]phenyl}propionic acid (IX) was obtained in 94% yield by hydrolysis of VIII with hot concentrated hydrochloric acid. An analytical sample, m.p. 85–86°, traveled as a single spot (R_f 0.72) on paper.[7] Chlorambucil had an R_f of 0.64 in the same system and could be detected in a concentration as low as one μg. Since no spot other than that with R_f 0.72 was detected when IX was run at 100 μg., the maximum amount of 3-[bis(2-chloroethyl)aminophenyl]propionic acid (a compound that would move more slowly (less polar) than IX) that could be present as an impurity in IX, would be 1%.

For the synthesis of the other "one-armed" mustard, 3-[*p*-(2-chloroethylamino)phenyl]propionic acid (X), the route II–V–VII–XI–X was successful. Tosylation of methyl 3-(*p*-aminophenyl)propionate yielded methyl 3-[*p*-(*p*-tolylsulfonamido)phenyl]propionate (V) in 84% yield, as a crystalline solid that traveled as a single spot (R_f 0.52) on paper.[7]

Attempts to hydroxyethylate V in the usual manner with ethylene oxide in aqueous acetic acid solution at room temperature failed to give any product, starting material being recovered. Heating V with ethylene oxide in benzene at 150° in a sealed tube gave a 93% yield of crude VI which could be purified by recrystallization from benzene–petroleum ether. This analytically pure VI moved as a single spot (R_f 0.61) on paper.[7]

Attempts to chlorinate VI using thionyl chloride in refluxing chloroform failed to yield any of the desired product, starting material and two other compounds, neither of which contained chlorine, being recovered. Therefore, VI was hydrolyzed to 3-[*p*-(2-hydroxyethylamino)phenyl]propionic acid (VIIa) using concentrated hydrochloric acid, followed by re-esterification with methanol. By this procedure, methyl 3-[*p*-(2-hydroxyethylamino)phenyl]propionate (VIIb) was obtained in 70% yield as a crystalline solid, m.p. 51–52°, that moved as a single spot (R_f 0.68) on paper.[7]

Several attempts to chlorinate VIIb using thionyl chloride in refluxing chloroform, followed by chromatography of the reduction products on acid-washed alumina, resulted only in intractable products containing variable amounts of chlorine. An analytical sample of methyl 3-[*p*-(2-chloroethylamino)phenyl]propionate (XI) was finally obtained in low yield by chlorination of VIIb as its hydrochloride with thionyl chloride in chloroform, followed by chromatography on acid-washed alumina and elution with chloroform. The product (XI) moved rapidly off the column and was shown to be homogeneous on paper[7] (R_f 0.60).

The low yields resulting from the action of thionyl chloride on the free base were primarily due to tar formation at the boiling point of chloroform. When the reaction was run at room temperature, it was too slow to be effective. However, when the chloride ion concentration was increased by addition of pyridine hydrochloride, the nucleophilic conversion of the intermediate chlorosulfite by the chloride ion to the chloroethyl derivative (XI) was greatly accelerated at room temperature compared with the rate of tar formation; XI was then isolated, after chromatography on acid-washed alumina, in 62% yield.

Hydrolysis of XI in concentrated hydrochloric acid by refluxing for one hour, followed by neutralization with sodium acetate, yielded 86% of 3-[*p*-(2-chloroethylamino)phenyl]propionic acid (X) as a colorless solid, m.p. 102–105°, that had R_f 0.66 on paper.[7]

Earlier attempts in these laboratories to prepare 3-[*p*-(2-hydroxyethylamino)phenyl]propionic acid (VIIa) *via* the method of Baxter and Cymerman-Craig,[9] *i.e.*, conversion of 3-(*p*-aminophenyl)propionic acid (IIa) or methyl 3-(*p*-aminophenyl)pro-

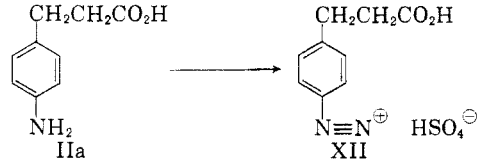
(7) Paper chromatograms were run by the descending technique on Schleicher and Schuell No. 2043B acetylated paper with benzene-methanol-water (6:2:1) as the solvent system. Compounds were detected by their ultraviolet absorption or by the use of *t*-butyl hypochlorite spray in the case of the monosubstituted amines.

(8) D. P. Schwartz and M. J. Pallansch, *Anal. Chem.*, **30**, 219 (1958).

(9) J. N. Baxter and J. Cymerman-Craig, *J. Chem. Soc.*, 1940 (1953).

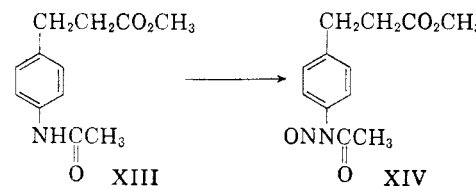
pionate (IIb) to the *N*-benzylidene derivative, quaternization with 2-bromoethanol, followed by acid hydrolysis of the quaternary salt to VIIa, failed.

3-(*p*-Diazoniumphenyl)propionic acid bisulfate (XII), another potential alkylating agent, was prepared in 64% yield by diazotization of 3-(*p*-aminophenyl)propionic acid using barium nitrite and sulfuric acid. Attempts to conduct the diazotization with sodium nitrite in hydrochloric acid



or 48% fluoboric acid[2] resulted in mixtures that could not be separated from the concomitantly formed inorganic salts. This difficulty was avoided in the barium nitrite–sulfuric acid diazotization by removing the insoluble barium sulfate by filtration prior to isolation of XII.

Methyl 3-[*p*-(*N*-nitrosoacetamido)phenyl]propionate (XIV) was prepared in 90% yield by the action of nitrosyl chloride on methyl 3-(*p*-acetamidophenyl)propionate (XIII) dissolved in glacial



acetic acid, in the presence of potassium acetate.[10] This compound (XIV) is stable in the solid state at 20° but decomposes at 0° in aqueous methanolic solutions.

Biological results.[11] Of the four alkylating agents, screening has been completed on all but X. Compounds IX, XII, and XIV showed no appreciable inhibiting effect at their maximum tolerated doses (9, 80, and 20 mg./kg., respectively) on Sarcoma 180, Adenocarcinoma 755, and Leukemia L-1210; chlorambucil is also noninhibitory on these three tumors. However, all three compounds produced a modest increase in survival time on mice bearing Ehrlich ascites, a tumor system that responds to chlorambucil. Compounds IX and XIV showed (+) activity, while compound XII had (±) activity.[12]

(10) H. France, I. M. Heilbron, and D. H. Hey, *J. Chem. Soc.*, 369 (1940).

(11) These tests were performed at this Institute by Dr. Joseph Greenberg and his staff under contract to the Cancer Chemotherapy National Service Center.

(12) H. J. Creech, T. S. Hauschka, F. F. Hankwitz, Jr., B. J. Littleton, and J. Andre, *Cancer Research*, Supplement No. 3, 47 (1955) have defined activity against Ehrlich ascites as (−) for 0–19%, a (±) for 20–50%, a (+) rating for 51–125%, a (++) rating for greater than 125% increase in survival time over untreated control animals.

EXPERIMENTAL[13]

Methyl 3-(p-ethylaminophenyl)propionate (I). To a solution of 1.79 g. (0.01 mole) of methyl 3-(*p*-aminophenyl)-propionate (IIb)[14] in 50 ml. of ethanol was added, dropwise with shaking, 0.88 g. (0.02 mole) of freshly distilled acetaldehyde. Anhydrous sodium acetate (0.1 g.) and 11 g. of Raney nickel were added and the mixture shaken with hydrogen at 56 p.s.i.g. for 4 hr. The mixture was filtered and the catalyst washed well with ethanol. The colorless filtrate was concentrated *in vacuo* to a sirup which was partitioned between water and ether. Concentration of the ether solution *in vacuo* gave a sirup which crystallized on rubbing. Recrystallization of this material from methanol-water gave a 61% yield of solid, m.p. 30–33°. Two more recrystallizations of this solid (0.5 g.) from two 1.5-ml. portions of methanol by addition of a few drops of water, gave analytically pure material, m.p. 35–36°. $\lambda_{max\ (\mu)}^{film}$ 2.97 (NH), 5.77 (ester C=O), 6.20, 6.57 (aryl, NH), 8.30, 8.61 (ester C—O—C), 12.25 (*p*-disubstituted phenyl). The compound traveled as a single spot on paper[7] (R_f 0.72), as detected by ultraviolet light or by development with a *t*-butyl hypochlorite spray[8] (violet color). The starting material (IIb) has R_f 0.68 in this system and developed a blue color with *t*-butyl hypochlorite.

Anal. Calcd. for $C_{12}H_{17}NO_2$: C, 69.5; H, 8.27; N, 6.76. Found: C, 69.2; H, 8.59; N, 6.73.

Methyl 3-{p-[ethyl(2-hydroxyethyl)amino]phenyl}propionate (IV). To a solution of 1.7 g. (8.3 mmoles) of methyl 3-*p*-ethylaminophenyl)propionate (I) dissolved in a solution of 10 ml. of glacial acetic acid and 10 ml. of water, was added 2 ml. of ethylene oxide with shaking. After 24 hr. at room temperature, the solution was poured into 40 ml. of water and neutralized with solid sodium hydrogen carbonate. The oil that separated was extracted with two 15-ml. portions of ethyl acetate. The combined extracts, dried over anhydrous magnesium sulfate, were filtered and concentrated *in vacuo* to a pale brown sirup; yield 1.87 g. (90%); $\lambda_{max\ (\mu)}^{film}$ 2.95 (OH), 5.73 (ester C=O), 8.35 (ester C—O—C), 9.55 (C—OH), 12.35 (*p*-disubstituted phenyl). The compound traveled as a single spot (R_f 0.83) on paper,[7] as detected by ultraviolet light. This sirup could not be crystallized, nor did it furnish a crystalline picrate.

Methyl 3-{p-[(2-chloroethyl)ethylamino]phenyl}propionate (VIII). To a solution of 1.4 g. (5.5 mmoles) of IV in 14 ml. of chloroform was added 0.60 ml. (8 mmoles) of thionyl chloride. After being refluxed for 15 min., the dark brown solution was poured onto ice; the chloroform layer was separated and dried over anhydrous magnesium sulfate. The filtered solution was concentrated *in vacuo* to about 5 ml., then chromatographed on a column of 20 g. of activated alumina (Merck acid-washed aluminum oxide). The product was eluted from the column with 30 ml. of chloroform, the pigmented impurities remaining adsorbed. Concentration of the pale yellow solution gave a light yellow sirup; yield 0.91 g. (61%); $\lambda_{max\ (\mu)}^{film}$ 5.72 (ester C=O), 8.30, 8.55, 9.65 (ester C—O—C), 12.33 (*p*-disubstituted phenyl), 13.60 (C—Cl), and no OH near 2.9. The sirup traveled as a single spot (R_f 0.79) on paper[7] as detected by ultraviolet light.

Anal. Calcd. for $C_{14}H_{20}ClNO_2$: C, 62.3; H, 7.43; Cl, 13.1; N, 5.19. Found: C, 62.6; H, 7.56; Cl, 13.3; N, 5.06.

3-[(2-Chloroethyl)ethylamino]phenyl propionic acid (IX).

(13) Melting points were taken on a Fisher-Johns block and are uncorrected.

(14) W. A. Skinner, H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 4639 (1959).

A solution of 0.85 g. (3.1 mmoles) of VIII in 8.5 ml. of concd. hydrochloric acid was refluxed for 2 hr. The dark solution was chilled and neutralized with saturated sodium acetate solution. The product, which crystallized on rubbing, was collected on a filter and washed with water: yield 0.72 g. (94%), m.p. 82–85°; $\lambda_{max\ (\mu)}^{KBr}$ 5.85 (acid C=O), 6.58 (aryl), 12.25 (*p*-disubstituted phenyl) 13.50 (C—Cl). The compound traveled as a single spot (R_f 0.72) on paper.[7] An analytical sample was prepared by two recrystallizations from ethanol-water, m.p. 85–86°.

Anal. Calcd. for $C_{18}H_{18}ClNO_2$: C, 61.1; H, 7.05; Cl, 13.9; N, 5.47. Found: C, 60.9; H, 7.16; Cl, 14.1; N, 5.45.

Methyl 3-[p-(p-tolylsulfonamido)phenyl propionate (V). To a solution of 1.79 g. (0.01 mole) of IIb[14] in 10 ml. of pyridine was added, with stirring in an ice bath, a solution of 2.86 g. (0.015 mole) of *p*-toluenesulfonyl chloride in 5 ml. of pyridine during a 10-min. period. The orange solution was stirred in ice for 3 hr., poured into 25 ml. of ice water, and extracted with three 10-ml. portions of ethyl acetate. The combined, dried solvent extracts were concentrated *in vacuo* to an orange sirup which was crystallized from methanol. The product was collected on a filter and washed with 50% aqueous methanol, yield 2.8 g. (84%), m.p. 83–87°; $\lambda_{max\ \mu}^{KBr}$ 3.10 (NH), 5.82 (ester C=O), 7.46 (—SO_2N—), 8.61 (ester C—O—C and —SO_2N—), 11.98, 12.24 (*p*-disubstituted phenyl). The compound traveled as a single spot (R_f 0.52) on paper.[7]

An analytical sample was prepared by three recrystallizations from methanol-water, m.p. 91–92°.

Anal. Calcd. for $C_{17}H_{19}NO_4S$: C, 61.2; H, 5.74; N, 4.20. Found: C, 61.5; H, 5.76; N, 4.08.

Methyl 3-{p-[N-(2-hydroxyethyl)-p-toluenesulfonamido]-phenyl}propionate (VI). To a solution of 1.0 g. (3 mmoles) of V in 10 ml. of benzene in a glass tube was added 5 ml. of ethylene oxide. The tube was sealed and heated in an oil bath at 150° for 5 hr. Concentration *in vacuo* gave a sirup that crystallized on standing. Recrystallization from benzene–petroleum ether gave 1.05 g. (93%), m.p. 94–96°.

An analytical sample was prepared by recrystallization from benzene–petroleum ether, m.p. 95–96°; $\lambda_{max\ (\mu)}^{Nujol}$ 2.88 (OH), 5.80 (ester C=O), 7.42 (—SO_2N—), 9.48 (C—OH), 12.25 (*p*-disubstituted phenyl). The compound moved as a single spot (R_f 0.61) on paper.[7]

Anal. Calcd. for $C_{19}H_{23}NO_5S$: C, 60.5; H, 6.14; N, 3.71. Found: C, 60.8; H, 6.22; N, 3.52.

A reaction time of 16 hr. gave a much lower yield.

Methyl 3-[p-(2-hydroxyethylamino)phenyl]propionate (VIIb). A suspension of 0.50 g. (1.3 mmoles) of VI in 5 ml. of concd. hydrochloric acid was heated under reflux for 1 hr. After 45 min., solution was almost complete; the mixture was decanted from a little oil and concentrated *in vacuo* to a pale brown sirup. This sirup (VIIa) was refluxed for 2 hr. in 20 ml. of methanol saturated with hydrogen chloride. The solution was concentrated *in vacuo* to a sirup, which was suspended in water and neutralized with 10% sodium hydrogen carbonate solution. The oil that separated was extracted with two 10-ml. portions of ethyl acetate and the combined extracts were dried over anhydrous magnesium sulfate. Concentration *in vacuo* of the extract freed from drying agent yielded 0.2 g. (70%) of a pale yellow sirup which crystallized on cooling. Recrystallization from benzene by addition of petroleum ether (b.p. 30–60°) yielded white platelets, m.p. 51–52°; $\lambda_{max\ (\mu)}^{Nujol}$ 3.00 (OH, NH), 5.78 (ester C=O), 6.20 (aryl), 6.55 (NH), 8.50, 8.65 (ester C—O—C), 9.50 (OH), 12.20 (*p*-disubstituted phenyl). The compound traveled as a single spot (R_f 0.68) on paper.[7]

Anal. Calcd. for $C_{12}H_{17}NO_3$: C, 64.5; H, 7.68. Found: C, 64.3; H, 7.67.

Methyl 3-[p-(2-chloroethylamino)phenyl]propionate (XI). A solution of 0.75 g. (3.3 mmoles) of VIIb in 8 ml. of chloroform was treated with hydrogen chloride. Then 1.1 g. (10 mmoles) of pyridine hydrochloride followed by 0.43 ml. (6 mmoles) of thionyl chloride, was added. The solution, protected from moisture, was allowed to stand for 16 hr. at room temperature and then refluxed for 10 min. The resultant solution was poured into ice water; the chloroform layer was separated, dried over anhydrous magnesium sulfate, and concentrated *in vacuo* to a light brown sirup (0.6 g.). This sirup was chromatographed on acid-washed alumina (20 g.) and eluted with chloroform, the yellow band being collected on elution. The eluate was a pale yellow sirup; yield, 0.5 g. (62%); $\lambda_{max\ (\mu)}^{film}$ 2.92 (NH), 5.72 (ester C=O), 8.30–8.60, 9.70 (ester C—O—C), 12.20 (*p*-disubstituted phenyl), 13.3–13.6 (C—Cl). The compound moved as a single spot (R_f 0.60) on paper.[7]

Anal. Calcd. for $C_{12}H_{16}ClNO_2$: C, 59.6; H, 6.67; Cl, 14.7; N, 5.79. Found: C, 59.9; H, 6.86; Cl, 14.3; N, 5.62.

3-[p-(2-Chloroethylamino)phenyl]propionic acid (X). A solution of 0.8 g. (3.3 mmoles) of XI in 8 ml. of concd. hydrochloric acid was refluxed for 1 hr., chilled, and neutralized with saturated sodium acetate solution. The precipitated solid was washed well with water and dried; yield 0.65 g. (86%), m.p. 102–105°. Three recrystallizations from 95% ethanol-water at room temperature yielded a sample with m.p. 103–105°; $\lambda_{max\ (\mu)}^{Nujol}$ 2.95 (NH), 5.81 (acid C=O), 7.65 (CO_2H), 12.18 (*p*-disubstituted phenyl), 13.80 (C—Cl). The compound moved as a single spot (R_f 0.66) on paper.[7]

Anal. Calcd. for $C_{11}H_{14}ClNO_2$: C, 58.0; H, 6.15; Cl, 15.6; N, 6.15. Found: C, 58.2; H, 6.17; Cl, 15.5; N, 6.20.

3-(p-Diazoniumphenyl)propionic acid bisulfate (XII). A solution of 4.98 g. (0.03 mole) of 3-(*p*-aminophenyl)propionic acid (IIa)[14] in 9 ml. of concd. sulfuric acid was cooled in an ice bath to 3° and diluted with 12 ml. of water. A solution of 3.95 g. (0.019 mole) of barium nitrite in 7 ml. of water at 3° was added with vigorous stirring to the acid solution over a 25-min. period. The reaction mixture was allowed to stand for 2 hr. at 0–5° and filtered. The barium sulfate residue was washed with 5 ml. of ice water and the filtrate evaporated *in vacuo* to about one-half the original volume while being chilled in ice. Fifty milliliters of cold ethanol was added followed by 150 ml. of ether until the solution was turbid. The product, which crystallized when the mixture was rubbed and cooled in a Dry Ice–acetone bath, was collected on a filter and thoroughly washed with 200 ml. of cold ether; yield 5.3 g. (64%), m.p. 94° dec.; $\lambda_{max\ (\mu)}^{Nujol}$ 3.75 (acidic OH), 4.40 ($N^+{\equiv}N$), 5.94 (acid C=O), 8.57, 9.50 (HSO_4^-), 12.05 (*p*-disubstituted phenyl and HSO_4^-).

Anal. Calcd. for $C_9H_{10}N_2O_6S$: C, 39.4; H, 3.67; N, 10.2. Found: C, 39.5; H, 3.97; N, 10.0.

Methyl 3-(p-acetamidophenyl)propionate (XIV). A mixture of 1.0 g. (0.0056 mole) of methyl 3-(*p*-aminophenyl)-propionate and 3 ml. of acetic anhydride was warmed for 5 min. on a steam bath and then chilled. The precipitate was washed with water and collected on a filter; yield 1.05 g. (84%), m.p. 119–123°; $\lambda_{max\ (\mu)}^{KBr}$ 3.09 (NH), 5.80 (ester C=O), 6.05 (amide C=O), 6.45 (amide NH), 11.94 (*p*-disubstituted phenyl). A sample, recrystallized twice from methanol-water, had m.p. 125–126°.

Anal. Calcd. for $C_{12}H_{15}NO_3$: C, 65.1; H, 6.83; N, 6.33. Found: C, 65.3; H, 6.91; N, 6.08.

Methyl 3-[p-(N-nitrosoacetamido)phenyl]propionate (XIII). To a solution of 6.66 g. (0.03 mole) of methyl 3-(*p*-acetamidophenyl)propionate (XIV) in 250 ml. of glacial acetic acid, 90 ml. of acetic anhydride, and 24 g. of anhydrous potassium acetate, stirred and cooled to 0–5°, was added dropwise a solution of 60 ml. of acetic anhydride and 15 ml. (0.3 mole) of nitrosyl chloride.[10] The reaction mixture was stirred 2 hr. at 0–5°, poured onto 1 kg. of ice, and the mixture was diluted with 1 l. of water. A yellow precipitate formed which was collected on a filter and washed thoroughly with ice water (about 1 l.); yield 6.8 g. (90%), m.p. 62.5–63.0° dec. For analysis, a portion of the yellow solid (0.3 g.) was recrystallized by dissolving it in 10 ml. of cold methanol and then adding 10 ml. of ice water; m.p. 62.5–63.0° dec.; $\lambda_{max\ (\mu)}^{Nujol}$ 5.75 (ester and amide C=O), 6.62, 6.78 (N=O), 8.45 (ester C—O—C), 11.98 (*p*-disubstituted phenyl).

Anal. Calcd. for $C_{12}H_{14}N_2O_4$: C, 57.6; H, 5.63; N, 11.2. Found: C, 57.4; H, 6.06; N, 11.4.

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MENLO PARK, CALIF.

[CONTRIBUTION FROM THE CANCER RESEARCH LABORATORY, DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF FLORIDA]

The Synthesis of 3-Deoxy-D-ribohexose-6-phosphate and 3-Deoxy-D-gluconic Acid-6-phosphate[1]

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3-Deoxy-D-ribohexose-6-phosphate and 3-deoxy-D-gluconic acid-6-phosphate have been prepared from 3-deoxy-D-ribohexose for testing as potential antimetabolites for cancer chemotherapy.

It is believed at the present time that glucose is metabolized in tumor tissue by two pathways, the Embden-Meyerhof glycolytic pathway, which is the main pathway quantitatively, and the pentose phosphate pathway, which apparently serves to supply reduced triphosphopyridine nucleotide (TPNH) for use in reductive syntheses.[4] There is evidence that the enzymes involved in the pentose phosphate pathway are present in greater amount in some tumor tissues than in normal tissues.[5]

Of the carbohydrate analogs which have been tested as glucose antagonists only those substituted in the 2-position have shown activity.[6] 2-Deoxy-D-glucose (2-deoxy-D-arabohexose) and 2-deoxy-D-galactose (2-deoxy-D-lyxohexose) are potent glycolytic inhibitors of human leucocytes, human leukemic cells, and a number of animal tumors.[7] Since the inhibition is competitive and is overcome by glucose-6-phosphate, it is apparently hexokinase, the enzyme which is necessary for the phosphorylation of glucose, which is affected.[7] This block occurs at a very early stage in the glycolytic pathway, before the pentose phosphate pathway begins to operate. It would be of interest, therefore, to prepare compounds having the potential ability to block the metabolic pathway at a later stage. This paper describes the preparation of two compounds which may have this potentiality, 3-deoxy-D-ribohexose-6-phosphate and 3-deoxy-D-gluconic acid-6-phosphate.

Using the procedure described by Reynolds and Evans[8] for the corresponding glucose derivative, 3-deoxy-D-ribohexose[9] was treated with triphenylmethyl chloride and then with acetic anhydride to obtain 1,2,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-3-deoxy-β-D-ribohexose (I). Upon treatment with hydrobromic acid in acetic acid the triphenylmethyl group was removed, giving 1,2,4-tri-*O*-acetyl-3-deoxy-β-D-ribohexose (II). Analysis showed this compound to be a monohydrate and the infrared spectrum had an absorption band at 1655 cm.$^{-1}$, indicating the presence of water. Attempts to remove the water azeotropically were only partially successful. To prove it was actually the 1,2,4-triacetate it was converted back to 1,2,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-3-deoxy-β-D-ribohexose (I).

The triacetate (II) was phosphorylated in the 6-position with diphenylphosphorochloridate using the procedure described by Lardy and Fischer[10] for the preparation of glucose-6-phosphate. The 1,2,4-tri-*O*-acetyl-3-deoxy-β-D-ribohexose-6-diphenylphosphate (III) was obtained as an oil. Low-pressure hydrogenation of (III) yielded 1,2,4-tri-*O*-acetyl-3-deoxy-β-D-ribohexose-6-phosphate (IV), also as an oil. Deacetylation in acid solution gave 3-deoxy-D-ribohexose-6-phosphate, which was isolated as the barium salt (V) and further purified as the brucine salt (VI).

Oxidation of the barium salt (V) was accomplished with barium hypoiodite by a modification of the procedure used by Levene and Raymond[11] for

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